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Dissection of a quantitative trait locus for genetic hypertension on rat chromosome 10

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ABSTRACT **We have previously identified a locus on rat chromosome 10 as carrying a major hypertension gene, *BP/SP-1*. The 100:1 odds support interval for this gene extended over a 35-centimorgan (cM) region of the chromosome that included the angiotensin I-converting enzyme (*ACE*) locus as demonstrated in a cross between the stroke-prone spontaneously hypertensive rat ($SHRSP_{HD}$) and the normotensive Wistar-Kyoto ($WKY\text{-}0_{HD}$) rat. Here we report on the further characterization of *BP/SP-1*, using a congenic strain, $WKY\text{-}1_{HD}$. $WKY\text{-}1_{HD}$ animals carry a 6-cM chromosomal fragment genotypically identical with $SHRSP_{HD}$ on chromosome 10, 26 cM away from the *ACE* locus. Higher blood pressures in the $WKY\text{-}1_{HD}$ strain compared with the $WKY\text{-}0_{HD}$ strain, as well as absence of linkage of the chromosome 10 region to blood pressure in an F_2 ($WKY\text{-}1_{HD}$ × $SHRSP_{HD}$) population suggested the existence of a quantitative trait locus, termed *BP/SP-1a*, that lies within the SHRSP-congenic region in $WKY\text{-}1_{HD}$. Linkage analysis in the F_2 ($WKY\text{-}0_{HD}$ × $SHRSP_{HD}$) cross revealed that *BP/SP-1a* is linked to basal blood pressure, whereas a second locus on chromosome 10, termed *BP/SP-1b*, that maps closer to the *ACE* locus cosegregates predominantly with blood pressure after exposure to excess dietary NaCl. Thus, we hypothesize that the previously reported effect of *BP/SP-1* represents a composite phenotype that can be dissected into at least two specific components on the basis of linkage data and congenic experimentation. One of the loci identified, *BP/SP-1a*, represents the most precisely mapped locus affecting blood pressure that has so far been characterized by random-marker genome screening.**

Human primary hypertension is one of the most common chronic diseases (1). It shows a significant degree of heritability (2–4) and is commonly recognized as a complex, polygenic disorder, with the exception of rare monogenetic forms (5, 6). The nature of complex disease makes it very difficult to identify contributing genes (7); one way to reduce the complexity of this challenge is the use of inbred animal models that exist for a number of human diseases, among them hypertension (8). Information about genetic factors identified in such experimental systems may provide insights into disease mechanisms that can then be applied to the generation and testing of hypotheses regarding the pathogenesis of the disease in humans.

By using random-marker genetic-screening methods in experimental crosses between the Heidelberg strains of the stroke-prone spontaneously hypertensive rat ($SHRSP_{HD}$) and the normotensive reference strain Wistar-Kyoto (WKY_{HD}), we previously identified a major quantitative trait locus for blood pressure (9, 10). The 100:1 odds interval for placing this locus, termed *BP/SP-1*, mapped to a region of rat chromosome 10 that spans 35 centimorgans (cM). This region was defined by simple-sequence length polymorphism (SSLP) markers from the genes coding for growth hormone (*RNGHGP*)¶, fast nerve growth factor receptor (*RNNGFRR*), and myosin heavy chain (*RNMHCG*). Linkage to *BP/SP-1* was highly significant for blood pressure values measured after dietary NaCl loading; the influence of this locus on baseline blood pressure was less significant and mapping therefore was less precise (Fig. 1).

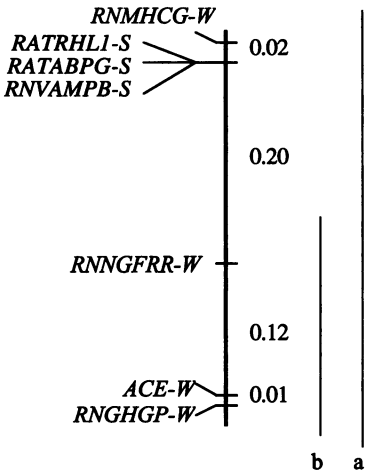

FIG. 1. Schematic of the linkage group based on published markers on chromosome 10 (9, 11) for the $WKY\text{-}1_{HD}$ genome. Lines a and b indicate the previously determined 100:1 odds support interval for placement of the blood pressure-relevant gene *BP/SP-1* for baseline and NaCl-induced blood pressure, respectively. "-W" denotes markers homologous with $WKY\text{-}0_{HD}$ alleles, whereas "-S" identifies markers identical to $SHRSP_{HD}$ alleles, defining the congenic region in $WKY\text{-}1_{HD}$. Recombination distances are given in centimorgans.

The linkage group containing these markers on chromosome 10 has been shown to be homologous to a region on human chromosome 17q23 that also carries the angiotensin I-converting enzyme (*ACE*) gene (12), an obvious candidate gene for hypertension (13).

The current set of experiments was aimed at further characterizing the *BP/SP-1* locus.

MATERIALS AND METHODS

Animals and Genetic Crosses. All animals were obtained from our colonies of $SHRSP_{HD}$, $WKY\text{-}0_{HD}$, and $WKY\text{-}1_{HD}$ rats at the University of Heidelberg (Heidelberg, Germany) (the subscript indicates this origin). $SHRSP_{HD}$ had been

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Abbreviations: SSLP, simple-sequence length polymorphism; cM, centimorgan(s).

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¶For clarity we have adopted the existing nomenclature for published rat genes as used in original publications and in GenBank.

obtained from the original Japanese stock from Okamoto and Aoki (14) in 1974 and propagated by strict inbreeding. WKY-0_{HD} and WKY-1_{HD} are two lineages of the WKY reference strain, also obtained from the Japanese stock in 1974, that were propagated as parallel families.

The first F_2 population used in the present study has been described in detail (15). All WKY animals that contributed to this cohort (n = 115) were from one WKY-0_{HD} litter, confirmed by genotype analysis. These animals were referred to as "WKY" in previous reports (9, 10, 15), since at that time the existence of the WKY-1_{HD} strain had not been recognized. For the second F_2 population reported in this paper, $SHRSP_{HD}$ rats were crossed with animals from one WKY-1_{HD} litter by following the same reciprocal breeding algorithm used in the previous study (15), thus generating an F_2 (WKY-1_{HD} × $SHRSP_{HD}$) cohort (n = 139). This F_2 population consisted of 33 males and 42 females with an $SHRSP_{HD}$ grandfather and 34 males and 30 females with a WKY-1_{HD} grandfather.

Phenotype Determination. Two different methods for blood pressure determination were used. Femoral artery cannulation and intermittent on-line recordings of tethered F_2 rats at baseline and after dietary NaCl loading were identical to the previously employed procedures (15).

A radiotelemetry method (Data Science International, Minneapolis) which allows highly accurate and reproducible blood pressure determinations was used to characterize parental WKY-0_{HD} (n = 8) and WKY-1_{HD} (n = 9) rats (16). The protocol for phenotype data sampling included measurements at baseline and after NaCl exposure in animals that were age-matched with the F_2 rats previously studied (9, 10).

DNA Sequencing. Comparative sequencing of genomic DNA from parental rat strains to identify polymorphisms was performed at several loci. PCR-derived amplification products from genomic DNA were used without further subcloning. PCR-amplified templates from each parental strain (WKY-0_{HD}, WKY-1_{HD}, and $SHRSP_{HD}$) were sequenced in duplicate with *Taq* dye-primer sequencing kits on an automated 373A DNA sequencer (Applied Biosystems).

Genotype Determination. The majority of polymorphic markers used were SSLPs; a few markers were represented by single base-pair differences (see below). SSLPs were obtained from the panel we used previously (9, 17) as well as from new library screenings (17) and amplified by PCR from 50 ng of genomic DNA in a final reaction volume of 10 μl, containing 100 nM each primer, 200 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris·HCl (pH 9.0 at 25°C), 0.1% Triton X-100, and 0.25 unit of *Taq* DNA polymerase. The forward primer was labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. PCR products were processed and subsequently analyzed by autoradiography after polyacrylamide gel electrophoresis as described (9, 17).

Chromosome 10 Marker Development. *Aldolase C (*RATALDCAA*) gene locus.* A $(GGT)_n$ repeat was identified in the published sequence of the *RATALDCAA* gene and was found to be informative for WKY-0_{HD} (allele size, 257 bp) and $SHRSP_{HD}$ (allele size, 255 bp) on rat chromosome 10 (18). Genotypes were determined by the procedure described above, using primers R422-F (5′-TACATAGTGAATAAACGGGAGC-3′) and R422-R (5′-TCAGGTAGGAGCCTGAGC-3′)—from positions 4371–4392 and 4610–4627, respectively, of the rat genomic sequence (18)—at an annealing temperature of 53°C.

*Acetylcholine receptor β subunit (*RNACRB1*) gene locus.* The *RNACRB1* gene was partially sequenced after amplification from genomic DNA using primers based on the rat mRNA sequence (11) and with reference to information available on the genomic structure of the mouse gene (19). By using primers RACRB4-F (5′-GGCGGTCCCCATCATCATCAAGTATC-3′) and RACRB4-R (5′-TTTGGGCCTCTTCAGACCCAGGTATG-3′) from positions 979–1004 and 1137–1162 of the rat cDNA sequences, respectively, an amplification product was obtained that carried a polymorphic $(CA)_n$ repeat (at the 3′ end of intron 18 by homology with the mouse, GenBank reference U27571). The flanking sequences of the repeat were determined as reported (20). WKY-0_{HD} (allele size, 226 bp) and $SHRSP_{HD}$ (allele size, 212 bp) genotypes were determined with primers RNACRB1-F (5′-ATTGTGGGCCACTACATC-3′) and RNACRB1-R (5′-ACAGCCATCTGTAAGTCTAAG-3′), applying an annealing temperature of 50°C.

*Na^+,K^+-ATPase β2 subunit (*RATATPB2S*) gene locus.* Comparative sequencing of PCR products that targeted the 5′ flanking region of the *RATATPB2S* gene (21) revealed a polymorphic site (T-for-C substitution in WKY-0_{HD}) at position 203 of the published sequence. This C-to-T transition resulted in a *Stu* I restriction fragment length polymorphism that was detectable by PCR using an annealing temperature of 70°C [forward primer, 5′-CCTGGCTGGCTTCTGTTGTTGTAA-3′; reverse primer, 5′-AGCCATGGCAGGTGACGTTAAGAT-3′; positions 30–53 and 574–597 (D90048 and M55328) of the reported sequence (21)]. Amplification products were digested with 10 units of *Stu* I (New England Biolabs) at 37°C for 4 hr and subsequently analyzed by electrophoresis in 2.0% agarose gels.

*Inducible nitric oxide synthase (*RATNOSI*) gene locus.* Genomic DNA sequence was generated in the 5′ flanking region of *RATNOSI* gene by using the available cDNA information (22). Amplification with primer pairs RATNOSI-1A (5′-CTCCTCAGGCTTGGGTCTTGTTAG-3′; positions 17–40 of the published sequence) and RATNOSI-1B (5′-GGTCACCTTGGTAGGATTTGACTC-3′; positions 131–154) generated a 1-kb fragment of genomic sequence with a polymorphic site in the parental rat strains (T in WKY-0_{HD} and C in $SHRSP_{HD}$) at position 136 of the genomic sequence (GenBank reference U27572). Genotypes were determined by allele-specific oligonucleotide hybridization. The polymorphic site was amplified by PCR using primers RATNOSI-1A and RATNOSI-2 (5′-CTCTTCCCACACCTTTCACATTAG-3′). The ASO probe (5′-TCCGCCCYGACCCTT-3′) was labeled with [γ-^{32}P]ATP as described above. The PCR product was diluted in denaturing solution and applied to duplicate nylon filters (GeneScreen; New England Nuclear) on a vacuum dot blot apparatus (Scotlab, Shelton, CT) as described (23). Prehybridization and hybridization were carried out at 41°C (23). The membranes were subjected to autoradiography for 6–12 hr.

Chromosomal Assignment and Placement of Markers. Chromosomal localization of all newly generated markers was ascertained with DNA from a panel of rat/mouse somatic cell hybrid lines kindly provided by T. Serikawa (17). Placement on the chromosomal map was carried out after genotype determination in the F_2 (WKY-0_{HD} × $SHRSP_{HD}$) cohort linkage analysis using the LINKAGE programs (24).

Statistical Analysis and Linkage Analysis. Statistical evaluation of the effects of a particular locus on phenotype in F_2 intercrosses was carried out by three-way ANOVA to account for genotype, sex, and parental constellation of the reciprocal crosses. For modeling two-gene interaction at the *BP/SP-1* locus, a dominant mode of inheritance was assumed and blood pressure values were adjusted for covariates (reciprocal cross and sex) by ANCOVA, as reported (9). All blood pressure values are expressed as mean ± SD.

RESULTS

Characterization of WKY-1_{HD} as a Congenic $SHRSP_{HD}$/WKY-0_{HD} Substrain. Among parallel lineages of WKY_{HD} rats maintained in the breeding facility, one was found by systematic genotype screening to carry $SHRSP_{HD}$-homologous alleles at markers *RATRHL1*, *RATABPG*, and *RATVAMPB* in place of the allelic forms previously seen in WKY-0_{HD} in a region of chro-

mosome 10 close to the *RNMHCG* locus (Fig. 1). We termed this substrain WKY1-$_{HD}$, to distinguish it from the WKY$_{HD}$ lineage used in earlier experiments, which we named WKY-0$_{HD}$. The *RNMHCG* and *RNNGFRR* markers, at a distance of 2 cM and 20 cM from *RATVAMPB* in opposite directions, were identical to the WKY-0$_{HD}$ alleles and thus still polymorphic with respect to the corresponding SHRSP$_{HD}$ alleles (Fig. 1). Additional screening of 296 SSLPs in these WKY-1$_{HD}$ rats revealed only one difference compared with WKY-0$_{HD}$—namely, partial heterozygosity for the SHRSP$_{HD}$ allele at marker RCA0709 on chromosome 3. Since one of the confines of the SHRSP-homologous region in WKY-1$_{HD}$ localized within the previously defined 100:1 odds interval for the placement of *BP/SP-1* (Fig. 1), we hypothesized that WKY-1$_{HD}$ may be congenic for the SHRSP$_{HD}$ allele of *BP/SP-1*. To test this, we obtained phenotype information on the WKY-1$_{HD}$ strain.

Phenotype Characterization of WKY-1$_{HD}$. Systolic and diastolic blood pressure values measured with radiotelemetry were significantly higher in WKY-1$_{HD}$ rats than in WKY-0$_{HD}$ rats when data were averaged over a 24-hr period both at baseline and after NaCl exposure (Table 1). Significant interstrain difference was also obtained when the analysis was performed separately for 12-hr periods during nighttime and daytime, respectively (data not shown).

Phenotype Characterization and Linkage Analysis in the F$_2$ (WKY-1$_{HD}$ × SHRSP$_{HD}$) Cross. We prepared a WKY-1$_{HD}$ × SHRSP$_{HD}$ cross to test the hypothesis that *BP/SP-1*, if contained within the SHRSP-homologous chromosome 10 region in WKY-1$_{HD}$, would not segregate in such a cross; this would result in overall higher blood pressures and in the inability to demonstrate linkage of blood pressure to *BP/SP-1*. Indeed, comparison of blood pressure values between the original F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) cross and the newly prepared F$_2$ (WKY-1$_{HD}$ × SHRSP$_{HD}$) cohort revealed significantly higher values in the latter among male and female animals, and before as well as after NaCl exposure (Fig. 2).

The values for basal blood pressure in the F$_2$ (WKY-1$_{HD}$ × SHRSP$_{HD}$) cross were similar to those measured in the F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) cross after NaCl loading; thus basal mean arterial blood pressures in both sexes were about 16 mmHg higher than in the F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) cross. At these higher baseline basal blood pressures, no differences were observed between reciprocal crosses, which is in keeping with the lack of such an effect among the original F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) cross after NaCl exposure (10). Moreover, as predicted by the working hypothesis, no linkage was detected between the markers on chromosome 10 that had previously exhibited significant linkage to blood pressure (9), indicating that *BP/SP-1* did in fact not segregate in this cross (Fig. 2).

Targeted Marker Development in the *BP/SP-1* Region. Efforts to more precisely define the congenic region resulted in the generation and mapping of additional, specifically targeted markers on chromosome 10 (Fig. 3). We identified two loci, *RNACRB1* and *RATATPB2S*, that showed identical alleles in WKY-1$_{HD}$ and SHRSP$_{HD}$ but were polymorphic with WKY-0$_{HD}$. Linkage analysis in the F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) cohort mapped these markers closely to *RATVAMPB*

Table 1. Phenotype comparison between WKY-0$_{HD}$ and WKY-1$_{HD}$

Phenotype	Blood pressure, mmHg*		*P* value (ANOVA)
	WKY-0$_{HD}$	WKY-1$_{HD}$	
Systolic basal	120.1 ± 2.7	124.3 ± 3.9	0.024
Diastolic basal	81.5 ± 1.3	86.1 ± 1.3	0.0009
Systolic NaCl	124.8 ± 3.1	131.4 ± 3.8	0.0018
Diastolic NaCl	83.5 ± 2.6	89.4 ± 1.9	0.0001

*One millimeter of mercury = 133 Pa.

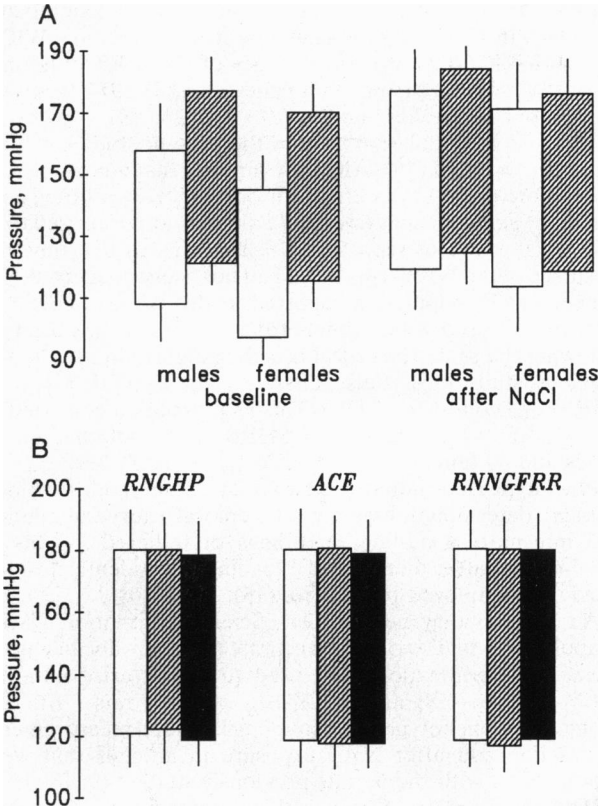

FIG. 2. Blood pressure phenotypes in the F$_2$ (WKY-1$_{HD}$ × SHRSP$_{HD}$) cross. (*A*) Comparison of overall systolic and diastolic blood pressures (upper and lower confines of bars, with standard deviation of the mean) between the F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) cross (open bars) and the F$_2$ (WKY-1$_{HD}$ × SHRSP$_{HD}$) cross (hatched bars). All values are significantly higher in the F$_2$ (WKY-1$_{HD}$ × SHRSP$_{HD}$) cross ($P < 0.0001$). (*B*) Blood pressures after NaCl loading in the F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) hybrids grouped by zygosity at three loci on chromosome 10 that showed significant linkage to this phenotype in the F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) cross. Open bars, *SS* homozygotes; hatched bars, *WS* heterozygotes; solid bars, *WW* homozygotes.

within the congenic region on chromosome 10 (Fig. 3). In contrast, two other newly developed markers representing the *RATALDCAA* and *RATNOSI* loci were polymorphic among WKY-0$_{HD}$ and SHRSP$_{HD}$, and identical in WKY-0$_{HD}$ and WKY-1$_{HD}$. They are closely spaced and map 6 cM away from *RNMHCG* and 17 cM from *RNNGFRR*. This allowed us to more precisely define the size and location of the SHRSP-congenic fragment of chromosome 10 in WKY-1$_{HD}$: the crossover points are localized in a 2-cM region between *RNMHCG* and *RATRHL1/RATABPG/RATVAMPB* and within a 3-cM distance between *RATALDCAA* and *RNACRB1*, narrowing the size of the congenic segment to a maximum of only 6 cM (Fig. 3).

Dissection of the *BP/SP-1* Locus into Two Components with Differential Phenotype Effects. Based on the newly established localization of the congenic region outside the previously demonstrated support interval for linkage to NaCl-induced blood pressure, but within the one defined for basal blood pressure, we reevaluated the data from the F$_2$ (WKY-0$_{HD}$ × SHRSP$_{HD}$) cross. Instead of using a single-locus, codominant model as in our previous study, we applied a two-locus model, assuming a dominant mode of inheritance, which we had previously demonstrated to be operative for *BP/SP-1* (9). This analysis revealed that while NaCl-loaded systolic blood pressure was strongly linked to the *RNGHGP* and *ACE* markers and mapped closely to these two genes within the previously characterized 20-cM-long odds interval (Fig. 1), this phenotype showed no significant linkage to markers within the

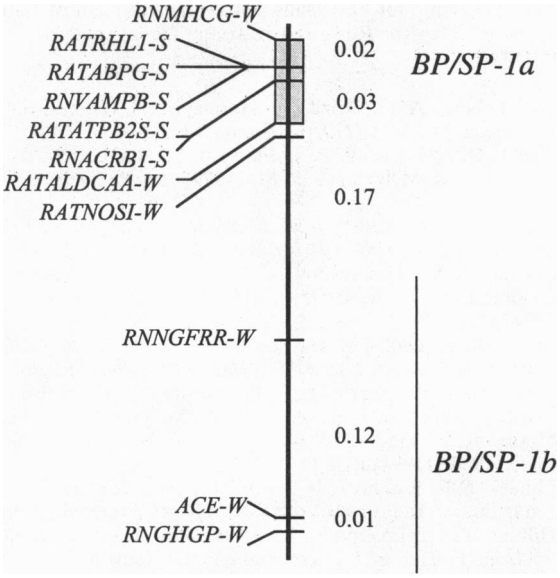

FIG. 3. Fine mapping of the congenic region (indicated by the box) on chromosome 10 in the WKY-1_{HD} genome by targeted marker development and dissection of *BP/SP-1* into two components, *BP/SP-1a* and *BP/SP-1b*. WKY-0_{HD} alleles (*-W*), $SHRSP_{HD}$-alleles (*-S*), and map distances are represented as in Fig. 1.

congenic region in WKY-1_{HD} (Table 2). Conversely, basal systolic blood pressure demonstrated significant cosegregation with markers in the congenic region (Table 2). These results suggest the existence of two, rather than one, blood pressure-relevant genes on chromosome 10 with different phenotype effects: *BP/SP-1a*, located within the congenic segment, appears to affect primarily baseline blood pressure, whereas *BP/SP-1b*, located within the previously identified confidence interval for NaCl-loaded blood pressure, seems to be more significantly linked to the NaCl-induced blood pressure phenotype (Fig. 3). The marker RCA0709 on chromosome 3, which showed partial heterozygosity for the $SHRSP_{HD}$ allele in WKY-1_{HD}, exhibited no significant effect on blood pressure in either the F_2 (WKY-0_{HD} × $SHRSP_{HD}$) or the F_2 (WKY-1_{HD} × $SHRSP_{HD}$) cross examined.

DISCUSSION

In this report we describe a series of studies conducted to further define our earlier findings of a blood pressure-relevant locus on chromosome 10 that segregates among the SHRSP and WKY strains. The present investigation suggests that the locus previously labeled *BP/SP-1* (9, 10) may in fact contain at least two separate genes, termed *BP/SP-1a* and *BP/SP-1b*, that segregate independently and confer differential phenotype characteristics.

The hypothesis that the *BP/SP-1a* locus contains a gene influencing blood pressure is supported by several observations. (*i*) The WKY-1_{HD} strain, which is homologous to $SHRSP_{HD}$ for *BP/SP-1a*, demonstrates significantly higher basal and NaCl-loaded blood pressure than WKY-0_{HD}. The difference between WKY-0_{HD} and WKY-1_{HD} in basal blood pressure represents about 50% of the difference between WKY-0_{HD} and $SHRSP_{HD}$ previously ascribed to *BP/SP-1*. (*ii*) We showed that the *BP/SP-1a* locus was significantly linked to basal systolic and diastolic blood pressure in the F_2 (WKY-0_{HD} × $SHRSP_{HD}$) cross, accounting for 24% and 22% of the variance of these phenotypes when data were analyzed adjusted for the *BP/SP-1b* effect and assuming a dominant model of inheritance. (*iii*) No linkage between the chromosome 10 region and blood pressure was detected in the F_2 (WKY-1_{HD} × $SHRSP_{HD}$) cross, indicating lack of segregation of a relevant genetic locus in this cross. (*iv*) Overall blood pressures in the F_2 (WKY-1_{HD} × $SHRSP_{HD}$) population were higher than in the F_2 (WKY-0_{HD} × $SHRSP_{HD}$) cross, consistent with the postulated homozygosity of all animals for the hypertensive (SHRSP-derived) allele at *BP/SP-1a* in the F_2 (WKY-1_{HD} × $SHRSP_{HD}$) cross. The relevance of the *BP/SP-1b* locus, on the other hand, is supported by its linkage to NaCl-loaded phenotypes in the F_2 (WKY-0_{HD} × $SHRSP_{HD}$) population. Cosegregation of this locus with blood pressure after NaCl exposure, although theoretically expected, was absent in the F_2 (WKY-1_{HD} × $SHRSP_{HD}$) cross, suggesting a less marked phenotypic effect of *BP/SP-1b* on the NaCl-induced blood pressure in the presence of overall higher basal blood pressure attributed to homozygosity at *BP/SP-1a*. Among other genetic loci previously shown to be linked to blood pressure (9, 10, 25) and tested in the F_2 (WKY-1_{HD} × $SHRSP_{HD}$) cross, the SA locus on chromosome 1 (25) was found to cosegregate with blood pressure, whereas a locus on chromosome 18 for which linkage to blood pressure had previously been suggested (10) could not be confirmed (data not shown).

The genealogy of the congenic strain used in the present studies, WKY-1_{HD}, has been reconstructed from the breeding history of the two strains $SHRSP_{HD}$ and WKY_{HD} at Heidelberg. Despite the fact that the WKY-1_{HD} strain was not deliberately bred, the origin of the introgressed chromosome 10 fragment in WKY-1_{HD} is almost certainly from $SHRSP_{HD}$, since only these two strains were maintained simultaneously in the breeding facility. This notion is furthermore confirmed by the fact that all genotypes within this region are identical to those found in $SHRSP_{HD}$, whereas other strains show different alleles at these loci (data not shown). The stable, homozygous presence of the introgressed SHRSP region in WKY-1_{HD} can be explained by a remote $SHRSP_{HD}$ × WKY-0_{HD} mating, followed by successive rounds of backcrossing onto WKY prior to the initiation of strict brother/sister mating, resulting in a

Table 2. Dissection of *BP/SP-1* into two components

Phenotype	Locus	Statistics by genotype, mmHg		ANOVA	
		S/S + W/S	*W/W*	*F*	*P*
Systolic basal	*BP/SP-1a*	150 ± 10 (n = 75)	145 ± 10 (n = 36)	8.5	<0.004
	BP/SP-1b	150 ± 10 (n = 83)	146 ± 11 (n = 28)	2.2	<0.14
Diastolic basal	*BP/SP-1a*	104 ± 8 (n = 75)	99 ± 9 (n = 36)	4.9	<0.029
	BP/SP-1b	104 ± 8 (n = 83)	98 ± 9 (n = 28)	7.5	<0.0072
Systolic NaCl	*BP/SP-1a*	178 ± 14 (n = 74)	171 ± 12 (n = 36)	2.7	<0.09
	BP/SP-1b	179 ± 13 (n = 82)	166 ± 11 (n = 28)	16.6	<0.0002
Diastolic NaCl	*BP/SP-1a*	121 ± 13 (n = 74)	115 ± 11 (n = 36)	1.6	<0.2
	BP/SP-1b	123 ± 12 (n = 82)	110 ± 11 (n = 28)	19.0	<0.0001

Blood pressure phenotypes (see text) at baseline and after NaCl loading in the F_2 (WKY-0_{HD} × $SHRSP_{HD}$) cross are grouped according to zygosity at the *BP/SP-1a* locus (as determined by the *RNACRB1* marker) and at the *BP/SP-1b* locus (as determined by the *ACE* marker). *S*, $SHRSP_{HD}$ allele; *W*, WKY-0_{HD} allele.

progressive dilution of SHRSP genetic material. Once strict inbreeding was begun (about 30 generations ago), part of the remaining heterozygous, SHRSP-derived material was lost, while the remainder became fixed in the homozygous state, establishing the WKY-1_{HD} lineage. As the original SHRSP admixture had gone unnoticed, and because the phenotype differences between WKY-0_{HD} and WKY-1_{HD} are easily demonstrated only with the use of sophisticated, high-fidelity instrumentation, there was no reason to suspect variability among WKY_{HD} lineages. The existence of the WKY-1_{HD} line was thus recognized only after the initiation of routine genotype screening for quality control purposes.

The portion of the $SHRSP_{HD}$-derived genetic material located on chromosome 10 spans a region of $\approx$6 cM or less between noncongenic markers. At present, the WKY-1_{HD} strain may therefore be viewed as representing a congenic line containing <1% SHRSP-derived genetic material introgressed into the WKY-0_{HD} genomic background (under the conservative assumption that the second $SHRSP_{HD}$-homologous marker on chromosome 3 indicates the presence of a congenic region similar in size to that on chromosome 10).

As in all congenic experimentation, the existence of additional genetic material derived from the "donor" strain at loci other than the targeted one is difficult to exclude. It is therefore possible that some of the effects observed in the present experiments, such as blood pressure difference among WKY-0_{HD} and WKY-1_{HD} or the lack of cosegregation of NaCl-loaded blood pressure with *BP/SP-1b*, may be related to SHRSP-congenic regions elsewhere in the WKY-1_{HD} genome that have so far escaped our attention (due to the less than ideally even distribution of genetic markers used). Such loci may affect blood pressure either directly or by interaction with chromosome 10 loci. The magnitude of the increase in basal blood pressure in $SHRSP_{HD}$ $\times$ WKY-1_{HD} hybrids over the basal pressure in rats derived from $SHRSP_{HD}$ $\times$ WKY-0_{HD} may indeed be viewed as supporting this notion. However, the possible existence of other congenic loci does not distract from the relevance of the congenic chromosome 10 region that is clearly evident from the striking difference between highly significant linkage to blood pressure in one cross and complete lack thereof in the other.

The identification of *BP/SP-1a* represents the most precise mapping of a gene contributing to hypertension reported so far in an experimental model starting from a genome screening approach (9, 10, 26). Further work on the establishment of additional congenic lines that carry smaller subfractions of the 6-cM-long congenic chromosome 10 region will be necessary to confirm *BP/SP-1a* and to reach a level of resolution that will make it feasible to begin physical mapping and (positional) cloning of its gene.

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